Early Onset Alzheimer’s Disease with Spastic Paraparesis,
Dysarthria and Seizures and N135S Mutation in PSEN1

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Abstract

Objective—Early onset familial Alzheimer’s disease (EOFAD) can be caused by mutations in genes for amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2). There is considerable phenotypic variability in EOFAD, including some patients with spastic paraparesis. The objective is to describe clinical and neuropathologic features of a family with a PSEN1 mutation that has been reported previously, without autopsy confirmation, in a single Greek family whose affected members presented with memory loss in their thirties, as well as variable limb spasticity and seizures.

Methods—We prospectively evaluated two children (son and daughter) with EOFAD and reviewed medical records on their mother. Archival material from the autopsy of the mother was reviewed and postmortem studies were performed on the brain of the daughter.

Results—All three individuals in this family had disease onset in their thirties, with cognitive deficits in multiple domains, including memory, language and attention, as well as less common features such as spastic dysarthria, limb spasticity and seizures. At autopsy both the mother and her daughter had pathologic findings of AD, as well as histological evidence of corticospinal tract degeneration. Genetic studies revealed a mutation in PSEN1 leading to an asparagine to serine substitution at amino acid residue 135 (N135S) in presenilin-1.

Conclusions—This is the first description of neuropathologic findings in EOFAD due to N135S PSEN1 mutation. The clinical phenotype was remarkable for spastic dysarthria, limb spasticity and seizures, in addition to more typical features of EOFAD.

Keywords
Alzheimer disease; Genetics; Neuropathology; Presenilin; Spasticity

Introduction

Autosomal dominant early onset familial Alzheimer disease (EOFAD) can be caused by mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1) or presenilin 2 (PSEN2) genes. The Alzheimer Disease and Frontotemporal Dementia Mutation Database

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(http://www.molgen.ua.ac.be/ADMutations) lists 455 families with 202 mutations in these three genes. Mutations in genes that cause EOFAD either increase the secretion of Aβ42, increase the ratio of Aβ42/Aβ40 or produce a more fibrillogenic form of Aβ, thus favoring amyloid deposition in the brain and providing supportive evidence for the amyloid cascade hypothesis of AD. It should be noted, however, that some EOFAD cases have no mutations in APP, PSEN1 or PSEN2, suggesting that other genes remain to be found. The PSEN1 gene encodes a protein that is an integral transmembrane protein made up of 467 amino acids and nine membrane-spanning domains. The PSEN1 protein is part of the γ-secretase complex, which is key in cleaving the APP protein into smaller proteins, including Aβ42 and Aβ40. Mutations in the PSEN1 gene increase the production of Aβ42, which is more prone to aggregate than Aβ40.

Patients with EOFAD show considerable phenotypic variability, and spastic paraparesis has been described in a number of patients with PSEN1 mutations. The aim of this report is to describe clinical and pathologic findings in a family with a PSEN1 mutation leading to an asparagine to serine substitution at amino acid residue 135 (N135S). This mutation has only been described in one family, without detailed clinical characterization or postmortem neuropathologic confirmation.

Methods

Clinical methods

The present study is based on retrospective review of medical records of the mother and prospective evaluations of her two children, a daughter and son. Evaluation of medical records was performed after informed consent of the legal next-of-kin and under the ethical guidelines of the Mayo Clinic Institutional Review Board. The prospective studies on the son and daughter included collection of clinical and family histories, physical and neurologic examinations, routine laboratory studies, neuropsychological testing and magnetic resonance imaging (MRI) of the brain.

Neuropathologic methods

Archival pathologic material from the autopsy of the mother was reviewed and a more detailed neuropathologic study was performed on the brain of the daughter. Archival glass slides from the mother had been stained with hematoxylin and eosin (H&E) and a few with Bielschowsky silver stains. Available were sections from cerebral cortex, hippocampus, amygdala, basal ganglia, substantia nigra, medulla and cerebellum. Subsequently, some of the slides were decolorized and used for immunohistochemistry for pan-Aβ (monoclonal antibody 13.3.3; 1:5000; Todd Golde, Mayo Clinic) and phospho-tau (CP13, 1:1000; Peter Davies, Albert Einstein College of Medicine) using standard laboratory methods and a DAKO Autostainer with Envision detection system. The section of medulla was decolorized and re-stained with immunohistochemistry for HLA-DR (LN-3, 1:100; ICN Biologicals, Lisle, IL) to detect activated microglia and macrophages and counterstained with Luxol fast blue to assess myelin integrity.

Gross examination and sampling of the daughter’s brain followed a systematic and standardized dissection protocol, including sampling from 6 regions of neocortex, anterior and posterior hippocampus, basal forebrain with amygdala, basal ganglia, thalamus, midbrain, pons, medulla, cervicomedullary junction, cerebellum and olfactory bulb. Dissected samples were embedded in paraffin and 5-µm thick sections were examined with hematoxylin and eosin (H&E). Sections of cortex, hippocampus, basal forebrain, basal ganglia and cerebellum were studied with thioflavin-S fluorescent microscopy. A section of the amygdala was immunostained for α-synuclein (polyclonal antibody NACP; 1:3000; Mayo Clinic...
Jacksonville). Additional cortical and brainstem sections were not stained for α-synuclein since the amygdala had no Lewy bodies or Lewy neurites, and since previous studies of a large number of AD cases had shown that in the absence of Lewy bodies in the amygdala, brainstem and cortical Lewy bodies are not found. Moreover, ubiquitin (Ubi-1; 1:5000; EnCor Biotechnology, Alachua, FL) immunohistochemistry of cortex, midbrain, medulla and cervicomedullary junction did not reveal any Lewy bodies. Sections of motor cortex, midbrain, medulla and spinal cord were stained with Luxol fast blue, HLA-DR and ubiquitin. Sections of cortex, hippocampus and basal forebrain were also studied with the Bielschowsky silver stain. Adjacent sections of cortex, hippocampus and cerebellum were immunostained for Aβ40 and Aβ42 (monoclonal antibodies 13.1.1 and 4.1.3; 1:000, after formic acid pretreatment; both antibodies from Todd Golde, Mayo Clinic, Jacksonville). A section of hippocampus was immunostained for TDP-43 (polyclonal; 1:3000, ProteinTech, Chicago, IL) according to a published protocol.21

Genetic methods

Genetic analysis was performed on DNA extracted from blood cells from both the son and daughter with polymerase chain reaction (PCR) and primers spanning all exons of PSEN1 as previously described.12, 22 The PCR products were sequenced using fluorescent dye-terminator chemistry in both directions according the manufacture directions (Applied Biosystems Inc, Foster City, CA).

Results

Clinical history of the mother

A Caucasian woman with two children developed first signs of cognitive impairment in her early thirties. This woman was adopted so it is not possible to determine if the mutation she carried was spontaneous or inherited. Both of her children had a similar disorder. She presented with impaired memory, and over a three-year period experienced gradual worsening of her intellectual facilities, such that she could no longer participate in her former activities, prepare meals or perform housework. She developed a seizure disorder four years prior to her death. Laboratory studies were within normal limits, and a brain biopsy showed changes consistent with AD. After 8 years of illness she died at 40-years-of-age. A complete autopsy was performed at an outside medical facility according to standard medical procedures and after consent from the legal next-of-kin.

Clinical history of the daughter

The patient’s daughter presented with memory problems that began at 32-years-of-age. She often repeated questions, forgot details of recent conversations and events, and routinely forgot to complete tasks assigned to her at work. At 35-years-of-age, her Mini Mental Status Examination23 (MMSE) score was 18 of 30. Neuropsychometric testing revealed a Mattis Dementia Rating Scale24 score of 109 of 144, indicating generalized cognitive difficulties. Moderate to severe impairment was seen in tests of divided attention, mental arithmetic, learning efficiency and recognition memory. Overall, she demonstrated a significant amnestic disturbance with impairment in learning, retaining new information, problem-solving skills and divided attention. Immediate attention, vocabulary and visuospatial skills were intact. MRI of the brain showed multiple small cerebral white matter lesions in the centrum semiovale as well as the periventricular white matter on T2 images consistent with small vessel disease (Fig. 1).

When she was evaluated at 37-years-of-age, she had prominent dysarthria, gait unsteadiness and agitation. She also had developed generalized tonic-clonic seizures. Her MMSE score had decreased to 10. Neurological examination revealed spastic dysarthria, spastic gait and

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symmetrical brisk reflexes in the biceps, triceps, brachioradialis, quadriceps, and gastrocnemius tendons. She also had a hyperactive jaw jerk and positive Hoffman signs. Four months later, she died and an autopsy limited to evaluation of the brain was performed.

**Clinical history of the son**

The patient’s son presented with memory difficulties that began at 33-years-of-age. He had 18 years of formal education, including a Masters in Business Administration, and he had previously worked as a marketing manager. At 40-years-of-age he reported increasing difficulty at work, noting impairment in the speed of thought processing, calculating, concentration, short term memory, and learning new information. He began to misplace objects, had trouble remembering names of people, and needed to take extensive notes as reminders to allow him to complete his work. He would repeatedly ask the same questions. He had no symptoms of depression, delusions or hallucinations. He developed dysarthria and balance difficulties, falling on a few occasions, and he reported gait unsteadiness when walking on uneven ground. He did not have impairment in basic activities of daily living, but he no longer drove a motor vehicle. He took donepezil and memantine.

His neurologic exam at 40-years-of-age revealed mild dysarthria, decreased rapid alternating movements on the left, mild difficulty with tandem gait and a MMSE of 24, with 0 of 3 on memory items. Neurological exam at 45-years-of-age was notable for a MMSE score of 22, with 6 of 10 on orientation and 0 of 3 on memory items. He had mild spastic dysarthria with decreased mouth rapid alternating movements for labials, linguals and palatals. He had brisk reflexes (legs more than arms) and mild spasticity in his legs. He walked with a mildly spastic, wide-based gait. Plantar responses were flexor on both sides, and there was no clonus. Fasciculations were absent. A jaw jerk reflex was present. He was able to do tandem gait with mild difficulty.

At 40-years-of-age his MRI, EEG and laboratory tests were unremarkable. Repeat MRI of the brain at age 45-years-of-age showed mild generalized atrophy, mild periventricular hyperintensities on T2 images consistent with small vessel disease, and bilateral (left more than right) enlargement of the temporal horns of the lateral ventricles seen on T1 images. There was left greater than right hippocampal atrophy (Fig. 1A).

**Neuropathologic findings of the mother**

The autopsy report and archival pathologic material were available for review on the mother. The autopsy had been performed 22 years before and only glass slides were available, the paraffin blocks having been discarded. The autopsy report described marked cortical atrophy, especially in the frontal regions, as well as marked loss of pigmentation of the substantia nigra. The ventricles were moderately enlarged, and the basal ganglia and thalamus were reported to be atrophic.

Sections of the cortex showed severe atrophy with marked neuronal loss, gliosis and status spongiosis. The cerebral white matter showed rarefaction with myelinated fiber loss and gliosis. Sections of cortex, hippocampus and basal ganglia showed numerous senile plaques and neurofibrillary tangles consistent with advanced AD (Fig. 2A). The Braak neurofibrillary tangle stage was consistent with Stage VI. The senile plaques showed a range of morphologic features, including diffuse plaques and plaques with dense amyloid cores. There was mild amyloid angiopathy in leptomeninges (Fig. 2B). Many of the senile plaques had neuritic elements, and there were numerous neuropil threads, especially with tau immunohistochemistry (Fig. 2C, 2D). The substantia nigra had neuronal loss and neurofibrillary tangles. There were amyloid deposits as well as Purkinje cell loss and Bergman gliosis in the cerebellum. The medullary pyramids showed findings consistent with wallerian
degeneration, characterized by vacuolation and decrease in myelinated fibers as well as myelin debris within the cytoplasm of macrophages (Fig. 3A, 3C). The findings contrasted with preservation in other tracts, such as the inferior cerebellar peduncle and the medial lemniscus (Fig. 3B, 3D).

**Neuropathologic findings of the daughter**

An autopsy limited to evaluation of the brain was performed on the daughter after consent from the legal next-of-kin. The brain was divided in the sagittal plane with the right half frozen for biochemical and genetic studies and the left half fixed in formalin for histologic studies. The calculated weight of the whole brain based upon the weight of the left half (510 grams) was 1020 grams. (The normal brain weight for adult women ranges from 1300 to 1100 grams.) There was mild cortical atrophy in superior frontal, inferior parietal and inferomedial temporal lobes. Coronal sections showed mild ventricular enlargement, particularly of the temporal horn of the lateral ventricle (Fig. 1B). Subcortical, brainstem and cerebellar structures were grossly unremarkable.

Microscopic examination of the neocortex revealed pathology that was less severe than her mother. There was minimal thinning of the cortical ribbon with focal gliosis and mild microvacuolation, usually associated with senile plaques, many of which were readily apparent on H&E due to dense amyloid cores (Fig. 4A). There were also many neuritic plaques (Fig. 4B) and numerous neurofibrillary tangles in the cortex, hippocampus (Fig. 4C) and basal nucleus of Meynert (Fig. 4D). The basal ganglia had many amyloid plaques, including neuritic type plaques and plaques with dense amyloid cores (Fig. 5A, 5B). There were also neurofibrillary tangles in the striatum. The Braak neurofibrillary tangle stage was consistent with Stage VI. There was mild focal amyloid angiopathy of the leptomeningeal vessels, but no significant arteriosclerotic vasculopathy. Both the hippocampus and entorhinal cortex had neuronal loss and gliosis. The substantia nigra had mild focal neuronal loss and neurofibrillary tangles, but no Lewy bodies. The cerebellum did not have significant Purkinje cell loss or Bergmann gliosis, but there were many diffuse amyloid deposits in the molecular layer. The amyloid deposits were mostly composed of Aβ42 with less Aβ40 (Fig. 6), especially diffuse amyloid plaques in cortex (Fig. 6A), hippocampus (Fig. 6B) and cerebellum (Fig. 6E). Amyloid in blood vessels and in plaques with dense amyloid cores had Aβ40 immunoreactivity (Fig. 6D, 6F).

The corticospinal tract showed mild degeneration, with mild rarefaction of the subcortical white matter beneath the motor cortex, as well as pallor and myelin vacuolation in the cerebral peduncles and medullary pyramid. The changes were less severe than in her mother, and were best appreciated by immunohistochemistry for activated microglia with HLA-DR (Fig. 7B). In contrast to the microgliosis in the corticospinal tract, there was no neuronal loss or microglial activation in the motor cortex (Fig. 7A) or in the hypoglossal nucleus (Fig. 7C, 7D). Immunohistochemistry for TDP-43, a marker for motor neuron disease, was negative.

**Genetic analyses of the son and daughter**

PCR analysis of DNA from the son and daughter revealed a point mutation at codon 135 of PSEN1 in the second transmembrane spanning domain, causing a change in the amino acid residue from asparagine to serine (N135S mutation). The codon is located at the beginning of the second transmembrane domain (Fig. 8). The mutation creates a DdeI restriction site, which was used to confirm the presence of the mutation in a separate PCR reaction.
Discussion

We evaluated a woman and her two children who had dementia beginning in their thirties. Notably, both children had an unusual phenotype for AD, with prominent spastic dysarthria and limb spasticity. Furthermore, the mother and daughter had seizures. The children were followed prospectively and had documented cognitive deficits in multiple domains, including memory, language and attention on formal neuropsychologic tests. The autopsy findings in the mother and daughter were those of AD and characterized by widespread neurofibrillary tangles and neuritic plaques, as well as mild amyloid angiopathy. While there were a few plaques that had the morphology of so-called "cotton wool plaques," which have been described in EOFAD due to PSEN1 mutations associated with spastic paraparesis, most of the senile plaques had prominent neuritic elements as well as dense amyloid cores, features that are absent in cotton wool plaques. The specificity of cotton wool plaques for PSEN1 mutations is not absolute, since they can be occasionally detected in sporadic Alzheimer’s disease. Moreover, EOFAD with extensive cotton wool plaques do not always have spastic paraparesis. In a review of the pathology of a large series of brains from patients with PSEN1 mutations, Mann and co-workers noted that there were two major types of amyloid deposition - one associated with deposits that had mostly Aβ42 immunoreactivity and the other with deposits showing almost equal Aβ40 and Aβ42 immunoreactivity as well as severe amyloid angiopathy. In type 1 many of the plaques in the cortex and especially the cerebellum are diffuse amyloid deposits, as in this case. The pathology in the present kindred is consistent with type 1, which was most often found in mutations in the amino-terminal half of presenilin, as in this case.

While the two children had documented spasticity and gait difficulties, this was not documented in the mother. Nevertheless, corticospinal tract pathology was detected in both the mother and her daughter, and the changes were actually more marked in the mother. Given the absence of clinical features in the mother, it raises the possibility that the observed changes in the pyramids may not be directly related to the observed long tract signs. It is also possible that long tract signs were present in the mother, but never documented given her severe cognitive problems. It is worth noting that only a few autopsy studies have documented corticospinal tract degeneration in EOFAD associated with spastic paraparesis, and that when it is detected the pathogenesis of tract degeneration is uncertain. It has been suggested that tract degeneration is linked to severe motor cortex pathology or to white matter pathology secondary to severe amyloid angiopathy. The motor cortex was not available for study in the mother. In the daughter the motor cortex had many senile plaques and neurofibrillary tangles, but no significant atrophy and relatively well preserved Betz cells. In neither the mother nor the daughter was there severe amyloid angiopathy. There was severe leukoencephalopathy in the mother in a distribution that paralleled cortical atrophy. The spinal cord was not available in either, but lower motor neurons in the hypoglossal nucleus were well preserved and there was no TDP-43 immunoreactivity. These findings argue against concurrent motor neuron disease as a cause of the tract degeneration as well as genetic evidence that has failed to identify variants in genes known to produce familial spastic paraparesis in EOFAD with spastic paraparesis.

This study is the first to demonstrate autopsy-confirmed Alzheimer’s disease and corticospinal tract degeneration in EOFAD with spastic paraparesis and seizures due to N135S mutation in PSEN1. A single family with this mutation has been previously reported, but there is no description of postmortem pathology in this family and only limited clinical information reported. In a multicenter survey of PSEN1 a novel Greek mutation was discovered in a family whose affected members presented with memory loss in their early-to-mid thirties and death from the disease in their early forties. Limb spasticity was reported in one family member and seizures in another. The clinical findings in our patients were similar to those reported in the Greek kindred, although spastic dysartrhia was not mentioned in the Greek family.
The clinical diagnosis of Alzheimer’s disease in the setting of early onset dementia with atypical clinical features, such as spasticity and spastic dystarthishia is challenging. Differentiating Alzheimer’s disease from one of the other disorders that can produce spastic paraparesis, such as frontal lobe degenerations,30 motor neuron disorders,31 hereditary vascular dementias,32 prion disorders 33 and other amyloidoses,34 requires clinical acumen as well as ancillary tests, including imaging and genetic testing. It recently has become possible to detect amyloid deposits in vivo with positron emission tomography, and these studies show early amyloid deposits in the basal ganglia in \textit{PSEN1} mutation carriers.35 It is thus of interest that the basal ganglia in the daughter also had many plaques and tangles. The most common genetic basis of autosomal dominant early onset dementia is linkage to chromosome 14 or presence of mutations in \textit{PSEN1}.37 Clinical heterogeneity is the rule in early onset dementia. In a review of very early onset AD, Snider and co-workers reported on a range of additional clinical features, including aphasia, seizures, pyramidal signs and parkinsonism.38 Similarly, in the large pathologic series reported by Mann and co-workers a similar spectrum of clinical features were noted in EOFAD due to \textit{PSEN1} mutations.27

The significance of mutation at codon 135 is that it is a residue that is shared by both \textit{PSEN1} and \textit{PSEN2} and, like other pathogenic mutations at codons 139, 143, and 147, is located within the transmembrane domain aligning on one face of an alpha helix (Fig. 6).39 This makes it likely that the \(\gamma\)-secretase activity is affected by this mutation.

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References

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Figure 1.
(A) MRI of the brain of the son (coronal view, T1 sequence) showing left > right hippocampal atrophy. (B) The coronal section of the brain of the daughter shows a similar appearance with mild atrophy that disproportionately affects the medial temporal lobe.
Figure 2.
A range of plaque types are detected in the brain of the mother, including some plaques with a so-called “cotton wool appearance” (A). Immunostaining for Aβ (B) shows mostly diffuse amyloid deposits, as well as mild focal amyloid angiopathy (inset). A Bielschowsky stain (C) shows neuritic elements in many of the plaques, as well as many cortical neurofibrillary tangles (inset). The tau immunostain (C) shows dystrophic neurites in plaques, but also profusely throughout the cortical gray matter. (A-D, ×400)
Figure 3.
Corticospinal tract degeneration in the medullary pyramid (A and C) compared to normal fiber tract in the adjacent medial lemniscus (B and D) on sections immunostained for HLA-DR for activated microglia (C and D) and counterstained with Luxol fast blue for myelin (A and B). Note vacuolated myelin and myelin debris in the cytoplasm of HLA-DR-positive macrophages (A) and well as hypertrophic microglia and macrophages (C) in the pyramid, but absence of these findings in the medial lemniscus (B and D). (A-D, ×400)
Figure 4. The senile plaques in the daughter (A and B) were readily apparent on routine H&E stains due to dense amyloid cores (arrows in A). The Bielschowsky stain shows prominent neuritic elements in many of the senile plaques (B). Inset shows higher magnification of a neuritic plaque. In the hippocampus (C) there are many neuritic plaques and flame-shaped neurofibrillary tangles. In the basal nucleus of Meynert (D) there are many globose neurofibrillary tangles (arrows) with Bielschowsky stain.
Figure 5.
Bielschowsky (A) and thioflavin-S (B) stained sections of the basal ganglia show many senile plaques, some of which have dense amyloid cores (inset). (A and B, ×400)
Figure 6.
Adjacent sections of cortex (A & B), hippocampus (C & D) and cerebellum (E & F) of the daughter immunostained for Aβ42 (A, C & E) and for Aβ40 (B, D & F). Note numerous cortical, hippocampal and cerebellar plaques, many appearing as diffuse deposits, with Aβ42, but only a few plaques with Aβ40 immunoreactivity. Even in those plaques with both (insets in A and B), there is more Aβ42 immunoreactivity. There is focal amyloid angiopathy in hippocampus and cerebellum. (A, B, E & F ×40; C & D × 100)
The motor cortex (A) and the hypoglossal nucleus (C and D) of the daughter show normal motor neurons (inset in A shows perineuronal vacuolation of Betz cells). Immunohistochemistry for HLA-Dr for activated microglia (B and D) showed microgliosis in the medullary pyramid (B), but not the hypoglossal nucleus (D). (A-D ×400)
Figure 8.
A model of the *PSEN* 1 protein showing 9 transmembrane spanning domains. Codon 135, (filled yellow circle at TM2 interface) which is the mutation in this kindred, has a serine substituted for an asparagine (N135S). The mutation is in the second transmembrane spanning domain, which serves as part of the active site of γ-secretase. Other pathogenic mutations in the second transmembrane spanning domain at codons 139 M, 143 I and 147 T align with codon 135 N on one face of an alpha helix.